

WHAT IS CLAIMED IS:

1. A gene which encodes reverse transcriptase having DNA polymerase activity and substantially no RNase H activity.

2. The gene of claim 1, wherein said gene is derived from an organism selected from the group consisting of Moloney murine leukemia virus (M-MLV), human T-cell leukemia virus type I (HTLV-I), bovine leukemia virus (BLV), Rous sarcoma virus (RSV), human immunodeficiency virus (HIV), yeast, Neurospora, Drosophila, primates and rodents.

3. The gene of claim 1, wherein said microorganism is M-MLV, comprising the following DNA sequence:

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      1078
ATG AOC CTA AAT ATA GAA GAT GAG CAT CGG CTA CAT GAG AOC TCA AAA GAG CCA GAT GGT
      1138
TCT CTA GGG TOC ACA TGG CTG TCT GAT TTT OCT CAG GOC TGG GCG GAA AOC GGG GGC ATG
      1198
GCA CTG GCA GTT GGC CAA GCT OCT CTG ATC ATA OCT CTG AAA GCA AOC TCT AOC CCC GTG
      1258
TOC ATA AAA CAA TAC CCC ATG TCA CAA GAA GOC AGA CTG GGG ATC AAG CCC CAC ATA CAG
      1318
AGA CTG TTG GAC CAG GGA ATA CTG GTA CCC TGC CAG TOC CCC TGG AAC ACG CCC CTG CTA
      1378
CCC GTT AAG AAA CCA GGG ACT AAT GAT TAT AGG OCT GTC CAG GAT CTG AGA GAA GTC AAC
      1438
AAG CGG GTG GAA GAC ATC CAC CCC AOC GTG CCC AAC OCT TAC AAC CTC TTG AGC GGG CTC
      1498
CCA CCG TOC CAC CAG TGG TAC ACT GTG CTT GAT TTA AAG GAT GOC TTT TTC TGC CTG AGA
      1558
CTC CAC CCC AOC AGT CAG OCT CTC TTC GOC TTT GAG TGG AGA GAT CCA GAG ATG GGA ATC
      1618
TCA GGA CAA TTG AOC TGG AOC AGA CTC CCA CAG GGT TTC AAA AAC AGT CCC ACC CTG TTT
      1678
GAT GAG GCA CTG CAC AGA GAC CTA GCA GAC TTC CGG ATC CAG CAC CCA GAC TTG ATC CTG
      1738
CTA CAG TAC GTG GAT GAC TTA CTG CTG GOC GOC ACT TCT GAG CTA GAC TGC CAA CAA GGT
      1798
ACT CGG GOC CTG TTA CAA AOC CTA GGG AAC CTC GGG TAT CGG GOC TGC GOC AAG AAA GOC
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CAA ATT TGC CAG AAA CAG GTC AAG TAT CTG GGG TAT CTT CTA AAA GAG GGT CAG AGA TGG 1858
 CTG ACT GAG GGC AGA AAA GAG ACT GTG ATG GGG CAG OCT ACT CCG AAG ACC OCT CGA CAA 1918
 CTA AGG GAG TTC CTA GGG ACG GCA GGC TTC TGT CGC CTC TGG ATC OCT GGG TTT GCA GAA 1978
 ATG GCA GGC CCC TTG TAC OCT CTC ACC AAA ACG GGG ACT CTG TTT AAT TGG GGC CCA GAC 2038
 CAA CAA AAG GGC TAT CAA GAA ATC AAG CAA GCT CTT CTA ACT GGC CCA GGC CTG GGG TTG 2098
 CCA GAT TTG ACT AAG CCC TTT GAA CTC TTT GTC GAC GAG AAG CAG GGC TAC GGC AAA GGT 2158
 GTC CTA ACG CAA AAA CTG GGA OCT TGG CGT CGG CCG GTG GGC TAC CTG TOC AAA AAG CTA 2218
 GAC CCA GTA GCA GCT GGG TGG CCC OCT TGC CTA CGG ATG GTA GCA GGC ATT GGC GTA CTG 2278
 ACA AAG GAT GCA GGC AAG CTA ACC ATG GGA CAG CCA CTA GTC ATT CTG GGC CCC CAT GCA 2338
 GTA GAG GCA CTA GTC AAA CAA CCC CCG GAC CGC TGG CTT TOC AAC GGC CGG ATG ACT CAC 2398
 TAT CAG GGC TTG CTT TTG GAC ACG GAC CGG GTC CAG TTC GGA CCG GTG GTA GGC CTG AAC 2458
 CCG GGT ACG CTG CTC CCA CTG OCT GAG GAA GGG CTG CAA CAC AAC TGC CTT GAT

or the degenerate variants thereof.

4. The gene of claim 1, wherein said micro-organism is M-MLV, comprising the following DNA sequence:

ATG ACC CTA AAT ATA GAA GAT GAG CAT CCG CTA CAT GAG ACC TCA AAA GAG CCA GAT GTT 1078
 TCT CTA GGG TOC ACA TGG CTG TCT GAT TTT OCT CAG GGC TGG GCG GAA ACC GGG GGC ATG 1138
 GGA CTG GCA GTT CGC CAA GCT OCT CTG ATC ATA OCT CTG AAA GCA ACC TCT ACC CCC GTG 1198
 TOC ATA AAA CAA TAC CCC ATG TCA CAA GAA GGC AGA CTG GGG ATC AAG CCC CAC ATA CAG 1258
 AGA CTG TTG GAC CAG GGA ATA CTG GTA CCC TGC CAG TOC CCC TGG AAC ACG CCC CTG CTA 1318
 CCC GTT AAG AAA CCA GGG ACT AAT GAT TAT AGG OCT GTC CAG GAT CTG AGA GAA GTC AAC 1378
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1438
AAG CGG GIG GAA GAC ATC CAC CCC ACC GIG CCC AAC CCT TAC AAC CTC TTG AGC GGG CTC

1498
CCA CCG TCC CAC CAG TGG TAC ACT GIG CTT GAT TTA AAG GAT GOC TTT TTC TGC CTG AGA

1558
CTC CAC CCC ACC AGT CAG OCT CTC TTC GOC TTT GAG TGG AGA GAT CCA GAG ATG GGA ATC

1618
TCA GGA CAA TTG ACC TGG ACC AGA CTC CCA CAG GGT TTC AAA AAC AGT CCC ACC CTG TTT

1678
GAT GAG GCA CTG CAC AGA GAC CTA GCA GAC TTC CGG ATC CAG CAC CCA GAC TTG ATC CTG

1738
CTA CAG TAC GIG GAT GAC TTA CTG CTG GOC GOC ACT TCT GAG CTA GAC TGC CAA CAA GGT

1798
ACT CGG GOC CTG TTA CAA ACC CTA GGG AAC CTC GGG TAT CGG GOC TCG GOC AAG AAA GOC

1858
CAA ATT TGC CAG AAA CAG GTC AAG TAT CTG GGG TAT CTT CTA AAA GAG GGT CAG AGA TGG

1918
CTG ACT GAG GOC AGA AAA GAG ACT GIG ATG GGG CAG OCT ACT CCG AAG ACC OCT CGA CAA

1978
CTA AGG GAG TTC CTA GGG ACG GCA GGC TTC TGT CGC CTC TGG ATC OCT GGG TTT GCA GAA

2038
ATG GCA GOC CCC TTG TAC OCT CTC ACC AAA ACG GGG ACT CTG TTT AAT TGG GGC CCA GAC

2098
CAA CAA AAG GOC TAT CAA GAA ATC AAG CAA GCT CTT CTA ACT GOC CCA GOC CTG GGG TTG

2158
CCA GAT TTG ACT AAG CCC TTT GAA CTC TTT GTC GAC GAG AAG CAG GGC TAC GOC AAA GGT

2218
GTC CTA ACG CAA AAA CTG GGA OCT TGG GGT CGG CCG GIG GOC TAC CTG TOC AAA AAG CTA

2278
GAC CCA GTA GCA GCT GGG TGG CCC OCT TGC CTA CGG ATG GTA GCA GOC ATT GOC GTA CTG

2338
ACA AAG GAT GCA GGC AAG CTA ACC ATG GGA CAG CCA CTA GTC ATT CTG GOC CCC CAT GCA

2398
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GTA GAG GCA CTA GTC AAA CAA CCC CCC GAC CGC TGG CTT TOC AAC GOC CGG ATG ACT CAC
2458
TAT CAG GOC TTG CTT TTG GAC ACG GAC CGG GTC CAG TTC GGA OCG GIG GTA GOC CTG AAC
2518
CCG GCT ACG CTG CTC CCA CTG CTT GAG GAA GGG CTG CAA CAC AAC TGC CTT GAT AAT TOC
2530
CGC TTA ATT AAT

or the degenerate variants thereof.

5. A gene which encodes a fusion protein which comprises reverse transcriptase having DNA polymerase activity and substantially no RNase H activity and a second protein comprising a hydrophobic leader peptide or a stabilizing peptide.

6. A vector containing the gene of claim 1 or 5.

7. The vector of claim 6 designated pRTdEcoRV-C which has been deposited at the American Type Culture Collection, Rockville Maryland under terms of the Budapest Treaty and given accession number 67555.

8. A host transformed with the vector of claim 6.

9. A polypeptide having an amino acid sequence encoded by the cloned gene of claim 1 or 5.

10. The polypeptide of claim 9 comprising the following amino acid sequence:

MET Thr Leu Asn Ile Glu Asp Glu His Arg Leu His Glu Thr Ser Lys Glu Pro Asp Val
 Ser Leu Gly Ser Thr Trp Leu Ser Asp Phe Pro Gln Ala Trp Ala Glu Thr Gly Gly MET
 Gly Leu Ala Val Arg Gln Ala Pro Leu Ile Ile Pro Leu Lys Ala Thr Ser Thr Pro Val
 Ser Ile Lys Gln Tyr Pro MET ser Gln Glu Ala Arg Leu Gly Ile Lys Pro His Ile Gln
 Arg Leu Leu Asp Gln Gly Ile Leu Val Pro Cys Gln Ser Pro Trp Asn Thr Pro Leu Leu
 Pro Val Lys Lys Pro Gly Thr Asn Asp Tyr Arg Pro Val Gln Asp Leu Arg Glu Val Asn
 Lys Arg Val Glu Asp Ile His Pro Thr Val Pro Asn Pro Tyr Asn Leu Leu Ser Gly Leu
 Pro Pro Ser His Gln Trp Tyr Thr Val Leu Asp Leu Lys Asp Ala Phe Phe Cys Leu Arg
 Leu His Pro Thr Ser Gln Pro Leu Phe Ala Phe Glu Trp Arg Asp Pro Glu MET Gly Ile
 Ser Gly Gln Leu Thr Trp Thr Arg Leu Pro Gln Gly Phe Lys Asn Ser Pro Thr Leu Phe
 Asp Glu Ala Leu His Arg Asp Leu Ala Asp Phe Arg Ile Gln His Pro Asp Leu Ile Leu
 Leu Gln Tyr Val Asp Asp Leu Leu Leu Ala Ala Thr Ser Glu Leu Asp Cys Gln Gln Gly
 Thr Arg Ala Leu Leu Gln Thr Leu Gly Asn Leu Gly Tyr Arg Ala Ser Ala Lys Lys Ala
 Gln Ile Cys Gln Lys Gln Val Lys Tyr Leu Gly Tyr Leu Leu Lys Glu Gly Gln Arg Trp
 Leu Thr Glu Ala Arg Lys Glu Thr Val MET Gly Gln Pro Thr Pro Lys Thr Pro Arg Gln
 Leu Arg Glu Phe Leu Gly Thr Ala Gly Phe Cys Arg Leu Trp Ile Pro Gly Phe Ala Glu
 MET Ala Ala Pro Leu Tyr Pro Leu Thr Lys Thr Gly Thr Leu Phe Asn Trp Gly Pro Asp
 Gln Gln Lys Ala Tyr Gln Glu Ile Lys Gln Ala Leu Leu Thr Ala Pro Ala Leu Gly Leu
 Pro Asp Leu Thr Lys Pro Phe Glu Leu Phe Val Asp Glu Lys Gln Gly Tyr Ala Lys Gly
 Val Leu Thr Gln Lys Leu Gly Pro Trp Arg Arg Pro Val Ala Tyr Leu Ser Lys Lys Leu
 Asp Pro Val Ala Ala Gly Trp Pro Pro Cys Leu Arg MET Val Ala Ala Ile Ala Val Leu
 Thr Lys Asp Ala Gly Lys Leu Thr MET Gly Gln Pro Leu Val Ile Leu Ala Pro His Ala
 Val Glu Ala Leu Val Lys Gln Pro Pro Asp Arg Trp Leu Ser Asn Ala Arg MET Thr His

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Tyr Gln Ala Leu Leu Leu Asp Thr Asp Arg Val Gln Phe Gly Pro Val Val Ala Leu Asn
Pro Ala Thr Leu Leu Pro Leu Pro Glu Glu Gly Leu Gln His Asn Cys Leu Asp.

11. The polypeptide of claim 9 comprising the following amino acid sequence:

MET Thr Leu Asn Ile Glu Asp Glu His Arg Leu His Glu Thr Ser Lys Glu Pro Asp Val
Ser Leu Gly Ser Thr Trp Leu Ser Asp Phe Pro Gln Ala Trp Ala Glu Thr Gly Gly MET
Gly Leu Ala Val Arg Gln Ala Pro Leu Ile Ile Pro Leu Lys Ala Thr Ser Thr Pro Val
Ser Ile Lys Gln Tyr Pro MET ser Gln Glu Ala Arg Leu Gly Ile Lys Pro His Ile Gln
Arg Leu Leu Asp Gln Gly Ile Leu Val Pro Cys Gln Ser Pro Trp Asn Thr Pro Leu Leu
Pro Val Lys Lys Pro Gly Thr Asn Asp Tyr Arg Pro Val Gln Asp Leu Arg Glu Val Asn
Lys Arg Val Glu Asp Ile His Pro Thr Val Pro Asn Pro Tyr Asn Leu Leu Ser Gly Leu
Pro Pro Ser His Gln Trp Tyr Thr Val Leu Asp Leu Lys Asp Ala Phe Phe Cys Leu Arg
Leu His Pro Thr Ser Gln Pro Leu Phe Ala Phe Glu Trp Arg Asp Pro Glu MET Gly Ile
Ser Gly Gln Leu Thr Trp Thr Arg Leu Pro Gln Gly Phe Lys Asn Ser Pro Thr Leu Phe
Asp Glu Ala Leu His Arg Asp Leu Ala Asp Phe Arg Ile Gln His Pro Asp Leu Ile Leu
Leu Gln Tyr Val Asp Asp Leu Leu Leu Ala Ala Thr Ser Glu Leu Asp Cys Gln Gln Gly
Thr Arg Ala Leu Leu Gln Thr Leu Gly Asn Leu Gly Tyr Arg Ala Ser Ala Lys Lys Ala
Gln Ile Cys Gln Lys Gln Val Lys Tyr Leu Gly Tyr Leu Leu Lys Glu Gly Gln Arg Trp
Leu Thr Glu Ala Arg Lys Glu Thr Val MET Gly Gln Pro Thr Pro Lys Thr Pro Arg Gln
Leu Arg Glu Phe Leu Gly Thr Ala Gly Phe Cys Arg Leu Trp Ile Pro Gly Phe Ala Glu
MET Ala Ala Pro Leu Tyr Pro Leu Thr Lys Thr Gly Thr Leu Phe Asn Trp Gly Pro Asp
Gln Gln Lys Ala Tyr Gln Glu Ile Lys Gln Ala Leu Leu Thr Ala Pro Ala Leu Gly Leu
Pro Asp Leu Thr Lys Pro Phe Glu Leu Phe Val Asp Glu Lys Gln Gly Tyr Ala Lys Gly
Val Leu Thr Gln Lys Leu Gly Pro Trp Arg Arg Pro Val Ala Tyr Leu Ser Lys Lys Leu

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Asp Pro Val Ala Ala Gly Trp Pro Pro Cys Leu Arg MET Val Ala Ala Ile Ala Val Leu
Thr Lys Asp Ala Gly Lys Leu Thr MET Gly Gln Pro Leu Val Ile Leu Ala Pro His Ala
Val Glu Ala Leu Val Lys Gln Pro Pro Asp Arg Trp Leu Ser Asn Ala Arg MET Thr His
Tyr Gln Ala Leu Leu Leu Asp Thr Asp Arg Val Gln Phe Gly Pro Val Val Ala Leu Asn
Pro Ala Thr Leu Leu Pro Leu Pro Glu Glu Gly Leu Gln His Asn Cys Leu Asp Asn Ser
Arg Leu Ile Asn.

12. A method of producing reverse transcriptase having DNA polymerase activity and substantially no RNase H activity comprising culturing the transformed host of claim 8 under conditions which produce reverse transcriptase, and isolating the reverse transcriptase so produced.

13. A method of preparing cDNA from mRNA, comprising

(a) contacting mRNA with an oligo(dT) primer or other complementary primer to form a hybrid, and

(b) contacting said hybrid formed in step (a) with reverse transcriptase, having DNA polymerase and substantially no RNase activity, and the nucleoside triphosphates to give a cDNA-RNA hybrid.

14. The method of claim 13, further comprising treating the cDNA-RNA with alkali or RNase H to selectively hydrolyze said RNA to give a cDNA.

15. The method of claim 13, further comprising treating said cDNA with DNA polymerase to give second-strand cDNA.

16. A kit for the preparation of cDNA from mRNA, comprising a carrier means being compartmentalized to receive in close confinement therein, one or more containers wherein

(a) a first container contains reverse transcriptase having DNA polymerase activity and substantially no Rnase H activity;

(b) a second container contains the nucleoside triphosphates, and

(c) a third container contains oligo(dT) primer.

17. The kit of claim 16, further comprising:

(d) a fourth container containing control RNA.

18. The kit of claim 16, wherein said second container further contains a buffer.

19. The kit of claim 16, wherein said reverse transcriptase is present at a concentration of 200 $\mu\text{g}/\mu\text{l}$ to 400 $\mu\text{g}/\mu\text{l}$.

20. The kit of claim 16, wherein said oligo (dT) primer is present at a concentration of 5 $\mu\text{g}/\text{ml}$ to 20 $\mu\text{g}/\text{ml}$.

21. The kit of claim 18, wherein said buffer comprises Tris-HCl (pH 7.5 to 8.3), KCl, MgCl_2 , and dithiothreitol.

23. The kit of claim 17, wherein said control RNA is present at a concentration of 10 $\mu\text{g/ml}$ to 20 $\mu\text{g/ml}$.

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